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Impact of UV-B Radiation on the Fecundity of the Copepod 'Acartia clausii'

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16. ABSTRACT

It has recently been demonstrated that acute midultraviolet irradiation (UV-B, 290 to 320 nm) of the marine copepod Acartia clausii results in reduced survival and fecundity. In the present study, immature late copepodites were separated by sex and exposed to three UV-B exposure levels (0, 25, and 50 effective $_{DNA}$ Jm^2). The irradiated copepods were then reared to sexual maturity and adult virgin survivors were mated according to prior exposure, supplying 7 different types of mating crosses (M0F0, M0F25, M25F0, M25F25, M0F50, M50F0, M50F50). Six replications of each cross were maintained for the duration of an experiment. Two experiments are documented. There was a significant effect of UV-B radiation on the survival of the parents as well as a reduction in the number of eggs and the number of living nauplii produced. For each experiment, 30 nauplii from each type of cross were separated, reated to maturity and counted, providing information on the survival capability of non-irradiated offspring from the seven different mating types.Previous exposure of parental stock did not significantly affect the survival, and development to maturity, of these nauplii 15 d after separation.

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Impact of UV-B Radiation on the Fecundity of the Copepod *Acartia clausii**

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Abstract

It has recently been demonstrated that acute midultraviolet irradiation (UV-B, 290 to 320 nm) of the marine copepod *Acartia clausii* results in reduced survival and fecundity. In the present study, immature late copepodites were separated by sex and exposed to three UV-B exposure levels (0, 25, and 50 effective$_{DNA}$ J m^{-2}). The irradiated copepods were then reared to sexual maturity, and adult virgin survivors were mated according to prior exposure, supplying 7 different types of mating crosses (M0F0, M0F25, M25F0, M25F25, M0F50, M50F0, M50F50). Six replications of each cross were maintained for the duration of an experiment. Two experiments are documented. There was a significant effect of UV-B radiation on the survival of the parents as well as a reduction in the number of eggs and the number of living nauplii produced. For each experiment, 30 nauplii from each type of cross were separated, reared to maturity and counted, providing information on the survival capability of non-irradiated offspring from the 7 different mating types. Previous exposure of parental stock did not significantly affect the survival, and development to maturity, of these nauplii 15 d after separation.

Introduction

The continued release of halocarbons into the atmosphere will, it is believed, eventually decrease the stratospheric ozone concentration. Recent findings predict the most probable value for the eventual ozone depletion due to continued release of chlorofluoromethanes at the 1977 level to be 16.5% (National Academy of Sciences, 1979). A 16.5% ozone reduction would result in approximately a 40 to 50% increase of biologically harmful, solar UV-B irradiance at mid-latitudes. Such radiation is readily absorbed by nucleic acids and proteins and is highly detrimental to biological systems. There is evidence that UV-B radiation, even at current levels, may have some effect on the survival of zooplankton in surface waters (Hunter *et al.*, 1979; Karanas *et al.*, 1979; Damkaer *et al.*, 1980). An enhancement of this stress, such as would result from ozone depletion, could possibly limit the development of a population.

The present work describes the effects of short-term, midultraviolet (UV-B, 290 to 320 nm) exposure on the subsequent reproduction of the marine copepod *Acartia clausii*. This species is a favorable assay animal, insofar as its sensitivity to UV-B radiation has been ascertained (Karanas *et al.*, 1979). *A. clausii* is also an abundant copepod in northern coastal and estuarine systems (Carrillo *et al.*, 1974) and has been the subject of much environmental research.

Previous study (Karanas *et al.*, 1979) demonstrated that the naupliar stages of *Acartia clausii* may be sensitive to the daily UV-B dose that could theoretically occur at depths inhabited by the organism. Copepodites and adults survived higher doses of UV-B radiation than did the nauplii. However, sublethal (usually subtle) effects can result in physiological or behavioral changes that may, over time, have a significant impact on the population. Many workers cite reproductive impairment as a sensitive indicator of sublethal stress (Marshall, 1962; Barnes and Barnes, 1968; Davis, 1972; Biesinger and Christiansen, 1972; Mitrovic, 1972; Reeve *et al.*, 1976, 1977a, b; Berdugo *et al.*, 1977; Lee, 1978; Ustach, 1979). Thus, effects on egg and naupliar production of A. clausii following exposure to current levels of UV-B radiation may be an important aspect of the copepod's adult biology.

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Materials and Methods

Specimens of *Acartia clausii* were collected at high tide from Yaquina Bay, Oregon (Station 21: 44°35'N; 124°01'W) the day prior to irradiation for two separate experiments during the summer of 1979. Immature late copepodites were separated by sex from these samples. Large numbers of the organisms were exposed to three UV-B dose levels (0, 25, and 50 effective$_{DNA}$ J m^{-2}), keeping the sexes separate at all times. Not all wavelengths within the UV-B waveband are equally effective in modifying the reproductive capability of *A. clausii*. The irradiance spectrum throughout the waveband differs between the radiation source used in this experiment and solar radiation at the earth's surface in the geographical regions where *A. clausii* is found. This dissimilar distribution of energy prevents direct comparison between the two radiation fields, for each mid-ultraviolet wavelength displays a distinctive ability in producing significant biological alterations. The shorter wavelengths possess a greater actinic nature than do the longer wavelengths. The sensitivity of an organism to the individual wavelengths of radiation comprising the UV-B region can be described by an action spectrum. Action spectra usually demonstrate the relationship between biological effectiveness and wavelength of monochromatic radiation and are specific for the endpoint of interest (in this case the reduced fecundity of *A. clausii*). Analytic representations of action spectra can then be used to weight the spectral irradiances for the two radiation regimes and allow their comparison. This approach presumes knowledge of the most applicable action spectrum, which is not known for *A. clausii*. However, Green and Miller (1975) have calculated an analytic representation of the long-wavelength tail of a DNA action spectrum compiled by Setlow (1974). This analytic equation was used to obtain weighting values for the exposure to each UV-B wavelength in the present study.

The organisms were irradiated in glass petri dishes (9 cm diam, 1.5 cm depth) containing 50 ml of 0.45 μm Millipore-filtered Yaquina Bay water collected from 1 m depth at the copepod sampling station.

Average site-water temperature and salinity recorded at the time of all collections were 16.2 °C ± 0.2 C° and 32.8 ± 1.2‰, respectively. Dishes were placed on a rotating turntable (16 rpm) beneath a lamp bank consisting of Vita-Lites® and Westinghouse FS20 sunlamps. Control organisms on the turntable were covered by 0.18 mm thicknesses of Mylar 'D'. Organisms to receive UV-B irradiation were covered by 0.25 mm thicknesses of 24 h photodegraded cellulose acetate for some portion of the experiment. The UV-B irradiance (290 to 320 nm) at the surface of the water was 0.583 W m^{-2} (5.28×10^{-3} eff$_{DNA}$ W m^{-2}) under cellulose acetate (Table 1). Under Mylar it was 0.028 W m^{-2} (1.68×10^{-6} eff$_{DNA}$ W m^{-2}). The visible irradiance (380 to 800 nm) during the exposure procedure was 9.80 W m^{-2}. The biologically effective irradiance used in this experiment is comparable to what would be expected to occur at solar noon on July 1 at the latitude where the copepods were collected (Green *et al.*, 1980; 0.296 cm ozone, 2.0 aerosol scaling coefficient).

Table 1. Absolute spectral irradiance in 290 to 320 nm waveband for the laboratory conditions (measured at water surface by a modified Gamma Scientific spectroradiometer) and for solar noon at Latitude 44°35'N on July 1 (0.296 cm ozone, 2.0 aerosol scaling coefficient; Green *et al.*, 1980)

Wavelength (nm)	Laboratory irradiance (W m^{-2} nm^{-1})	Global irradiance (W m^{-2} nm^{-1})
290	1.98×10^{-5}	3.00×10^{-6}
292	2.12×10^{-4}	3.40×10^{-5}
294	1.63×10^{-3}	2.41×10^{-4}
296	5.52×10^{-3}	1.17×10^{-3}
298	1.04×10^{-2}	4.16×10^{-3}
300	1.48×10^{-2}	1.14×10^{-2}
302	2.06×10^{-2}	2.55×10^{-2}
304	2.31×10^{-2}	4.81×10^{-2}
306	2.53×10^{-2}	7.97×10^{-2}
308	2.75×10^{-2}	1.19×10^{-1}
310	2.83×10^{-2}	1.64×10^{-1}
312	2.78×10^{-2}	2.13×10^{-1}
314	2.74×10^{-2}	2.62×10^{-1}
316	2.69×10^{-2}	3.11×10^{-1}
318	2.66×10^{-2}	3.59×10^{-1}
320	2.67×10^{-2}	4.03×10^{-1}

The temperature range to which copepods were exposed during separation and irradiation never extended beyond the organism's optimal thermal range. To permit easy separation and handling of copepodites after collection, the water temperature was lowered to 6 °C ± 1.0 C° over a 60 min period. The copepods sluggish, but motion always occurred at these temperatures. Prior to irradiation, the water temperature was raised to the initial temperature of the irradiation area (13.1 °C ± 0.5 C°) over a second 60 min period. Finally, the water temperature gradually increased to a maximum of 16.3 °C ± 0.5 C° during the irradiation procedure.

The irradiated copepods were reared to sexual maturity at a temperature of 13.0 °C ± 0.5 C°, maintaining the separation between sexes and treatments. Previous experimentation indicated that exposure to these doses would result in approximately a 25% reduction of the population size. In the present study survivor counts at the end of 5 d confirmed this.

After the 5 d holding period, adult virgin survivors were mated according to exposure, providing 7 different types of mating crosses (M0F0, M0F25, M25F0, M25F25, M0F50, M50F0, M50F50; e.g. M0F25 denotes the male having received 0 eff$_{DNA}$ J m^{-2} and the female 25 eff$_{DNA}$ J m^{-2}). Six replications of each cross were maintained for the duration of an experiment. The mated pairs were placed in 150 ml beakers containing 140 ml of the Millipore-filtered Yaquina Bay water with 37 mg l^{-1} EDTA. Phytoplankton provided as food were *Dunaliella tertiolecta* and *Isochrysis galbana* in a total concentration of about 2×10^5 cells ml^{-1}. A ciliate, *Euplotes* sp., was introduced for bacterial control. The experimental design was such that

enough copepods were initially irradiated to provide a substantial surplus of both sexes for all treatments for later use. Each mating pair was maintained in a separate non-aerated 150 ml vessel for two 5 d intervals (the copepods were transferred to new medium on the fifth day). Evidence has been presented for species of *Acartia* which describes the fluctuation of fecundity in the laboratory with variables introduced through standardized culture, i.e., effects of continuous and temporary starvation, and presence or absence of male copepods (Parrish and Wilson, 1978). More stable egg-laying patterns were observed for females where there was a constant food supply and the continued presence of a male copepod. Therefore, experimental vessels were monitored every second day for fluctuations in food concentration and welfare of organisms. Ordinarily, there was a noticeable decrease in the food concentration before 5 d (and transfer to new medium). Phytoplankton concentration was adjusted to the original level whenever depletion was detected. Males that died were replaced at any time during the experiment. Females that died within the first 5 d were replaced and data collection for that pair was started anew. When females died during the second 5 d period, data for that pair were disregarded. After 10 d, the parental pair was removed from the mating vessels and 2 d later (the time necessary for fertilized eggs of *A. clausii* to hatch at 13 °C; Landry, 1976) the number of living nauplii, dead nauplii and unhatched eggs were recorded. For each cross, 30 of the living nauplii produced by the 6 replications were separated ,reared to maturity and the survivors counted (15 d past separation). These samples provided information on the survival of non-irradiated offspring from the 7 different parental mating types.

All irradiated organisms and subsequent progeny were maintained in water-cooled trays. Food concentration and temperature were closely controlled in this experiment due to their high degree of correlation with fecundity. Water-bath temperatures (13.0 °C ±0.5 C°) were slightly lower than temperatures recorded at the sample site. Because of lack of mixing in the culture vessels, only flagellated phytoplankton known to satisfy the nutritional needs of all copepod life stages were provided. Also, to avoid any display of phototatic behavior by the organisms, only diffuse light (380 to 800 nm) was provided (9.15×10^{-3} W m^{-2}).

Results

The survival of male and female late copepodites of *Acartia clausii*, irradiated (5.28×10^{-3} eff_{DNA} W m^{-2}) one day after field collection, for two separate experiments, is recorded in Table 2 and illustrated in Fig. 1 for the 0 to 50 eff_{DNA} J m^{-2} dose range. The mean surviving fraction for each treatment normalized by the average control surviving fraction is depicted in Fig. 1 with 95% confidence intervals. Two-way ANOVA between replications and treatments for survival data indicate a significant difference in the survival of the organisms following different doses of UV-B radiation ($P < 0.05$) and a non-significant difference between the two separate experiments ($P > 0.05$). Replications were combined for estimation of surviving fractions. Also included in Fig. 1 are survival responses of late copepodites and adults for a wider dose range where an effective UV-B irradiance of 1.13×10^{-2} eff_{DNA} W m^{-2} was used (Karanas *et al.*, 1979). Least-squares regression analy-

Table 2. *Acartia clausii.* Late copepodite survival data for two experiments. Data are shown for each sex and treatment group ($n = 60$ per group)

Total dose (eff_{DNA} J m^{-2})	Percentages of males surviving		Percentages of females surviving	
0	95	98	85	100
25	72	73	82	77
50	67	67	70	73

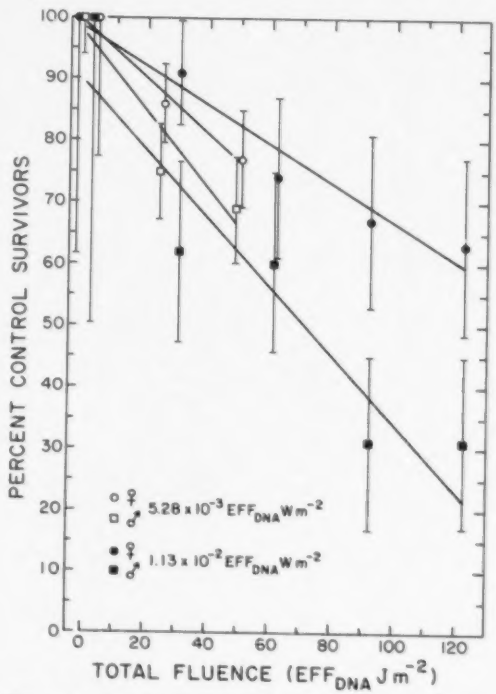

Fig. 1. *Acartia clausii.* Least-squares regression analysis illustrating survival of male and female late copepodites in response to two different DNA-effective UV-B irradiance levels (raw data for 1.13×10^{-2} eff_{DNA} W m^{-2} regressions obtained from Karanas *et al.*, 1979). Mean surviving fraction normalized by average control surviving fraction is shown for each treatment with 95% confidence intervals. Regression equations (where y is surviving number and x is dose) are as follows:

Irradiance	Sex	Regression
5.28×10^{-3} eff_{DNA} W m^{-2}	♀	$y = 0.989 - 0.0045\,(x)$
	♂	$y = 0.968 - 0.0062\,(x)$
1.13×10^{-2} eff_{DNA} W m^{-2}	♀	$y = 0.985 - 0.0032\,(x)$
	♂	$y = 0.890 - 0.0055\,(x)$

Table 3. *Acarti clausii.* Living nauplii, dead nauplii, and unhatched eggs produced by all replicates of the 7 different types of mating crosses of irratiated copepods (first experiment). Each column represents results obtained for one 5 d period

Mating pair	Living nauplii		Dead nauplii		Unhatched eggs		Total living (SD)		Total production (SD)	
$M0F0_1$	22	13	0	3	2	3	35		43	
$M0F0_2$	39	18	0	0	0	1	57		58	
$M0F0_3$	58	14	1	0	6	0	72	$\bar{x}=54.33$	79	$\bar{x}=61.00$
$M0F0_4$	27	22	1	0	0	4	49	(± 13.23)	54	(± 13.30)
$M0F0_5$	45	20	0	2	3	4	65		74	
$M0F0_6$	29	19	2	5	1	2	48		58	
$M0F25_1$	29	7	5	0	3	6	36		50	
$M0F25_2$	15	4	0	1	13	5	19		38	
$M0F25_3$	8	10	0	6	17	5	18	$\bar{x}=23.83$	46	$\bar{x}=50.50$
$M0F25_4$	13	19	5	10	14	5	32	($\pm$ 8.38)	66	($\pm$ 9.29)
$M0F25_5$	10	5	4	2	10	18	15		49	
$M0F25_6$	17	6	4	0	26	1	23		54	
$M25F0_1$	15	10	1	0	16	7	25		49	
$M25F0_2$	12	2	4	0	13	9	14		40	
$M25F0_3$	7	3	0	6	32	11	10	$\bar{x}=20.50$	59	$\bar{x}=52.83$
$M25F0_4$	21	8	2	4	13	9	29	($\pm$ 8.22)	57	($\pm$ 8.75)
$M25F0_5$	18	11	4	10	11	10	29		64	
$M25F0_6$	14	2	3	3	12	14	16		48	
$M25F25_1$	13	6	0	7	31	9	19		66	
$M25F25_2$	25	1	2	1	20	8	26		57	
$M25F25_3$	15	6	1	3	47	15	21	$\bar{x}=16.67$	87	$\bar{x}=57.00$
$M25F25_4$	6	4	0	0	18	4	10	($\pm$ 6.80)	32	(± 18.63)
$M25F25_5$	8	8	4	2	17	7	16		46	
$M25F25_6$	5	3	8	1	27	10	8		54	
$M0F50_1$	17	0	6	4	22	8	17		57	
$M0F50_2$	0	0	0	0	20	15	0		35	
$M0F50_3$	8	1	2	0	14	18	9	$\bar{x}=8.17$	43	$\bar{x}=45.33$
$M0F50_4$	0	0	2	0	24	12	0	(± 9.99)	38	(± 10.17)
$M0F50_5$	22	1	4	0	30	2	23		59	
$M0F50_6$	0	0	0	2	33	5	0		40	
$M50F0_1$	7	0	4	3	32	14	7		60	
$M50F0_2$	0	0	2	0	34	18	0		54	
$M50F0_3$	6	0	3	1	27	15	6	$\bar{x}=7.50$	52	$\bar{x}=48.33$
$M50F0_4$	0	1	0	3	10	12	1	(± 7.12)	26	(± 11.74)
$M50F0_5$	12	0	0	0	25	10	12		47	
$M50F0_6$	15	4	0	5	16	11	19		51	
$M50F50_1$	2	0	0	0	0	12	2		14	
$M50F50_2$	0	2	0	0	26	13	2		41	
$M50F50_3$	0	0	0	3	31	7	0	$\bar{x}=6.83$	41	$\bar{x}=38.83$
$M50F50_4$	9	1	3	7	11	9	10	(± 6.40)	40	(± 15.35)
$M50F50_5$	16	0	0	0	14	5	16		35	
$M50F50_6$	10	1	7	2	29	13	11		62	

sis was utilized to compare the organism's survival response with dose for each sex and for the two irradiance levels used. All slopes are significant ($P < 0.01$), further corroborating the sensitivity of the organism to UV-B radiation.

The use of field individuals for the more recent study provided the opportunity to determine whether their sensitivity differs from that of laboratory organisms reared without a UV-B component in their daily illumination (as in Karanas *et al.*, 1979). A comparison of regressions between the present survival experiment and those previously recorded (Karanas *et al.*, 1979; data illustrated in Fig. 1) indicates a non-significant difference ($P > 0.05$) in the survival response of either male or female late copepodites irradiated soon after field collection or after laboratory cultivation. These comparisons also suggest non-significant differences in the response of these organisms to the two irradiance levels employed. Non-significant differences ($P > 0.05$) were found between male and female regressions in the more recent experiment (using field individuals), in contrast with previous documentation (Karanas *et al.*, 1979); however, the range of exposure was far smaller in the present experiment.

Tables 3 and 4 present the data obtained from two independent fecundity experiments. Six replications of the 7 types of mating crosses were maintained for each. Ob-

Table 4. *Acartia clausii.* Living nauplii, dead nauplii, and unhatched eggs produced by all replicates of the 7 different types of mating crosses of irradiated copepods (second experiment). Each column represents results obtained for one 5 d period

Mating pair	Living nauplii		Dead nauplii		Unhatched eggs		Total living (SD)		Total production (SD)	
$M0F0_1$	25	36	3	6	3	5	61		78	
$M0F0_2$	48	13	1	0	4	3	61		69	
$M0F0_3$	24	25	1	1	2	4	49	$\bar{x}=56.83$	57	$\bar{x}=66.33$
$M0F0_4$	39	31	6	2	0	2	70	($\pm$ 9.91)	80	($\pm$12.91)
$M0F0_5$	15	43	0	2	1	7	58		68	
$M0F0_6$	19	23	2	0	2	0	42		46	
$M0F25_1$	24	3	2	3	5	7	27		44	
$M0F25_2$	10	22	0	6	9	11	32		58	
$M0F25_3$	14	4	7	1	8	20	18	$\bar{x}=26.50$	54	$\bar{x}=53.33$
$M0F25_4$	30	8	0	2	14	9	38	($\pm$ 7.45)	63	($\pm$ 6.68)
$M0F25_5$	18	5	0	3	15	11	23		52	
$M0F25_6$	16	5	12	2	7	7	21		49	
$M25F0_1$	22	16	1	2	12	8	38		61	
$M25F0_2$	9	1	10	4	17	4	10		45	
$M25F0_3$	29	18	6	2	2	8	47	$\bar{x}=28.83$	65	$\bar{x}=55.17$
$M25F0_4$	8	19	3	5	14	9	27	($\pm$13.26)	58	($\pm$13.60)
$M25F0_5$	13	6	10	2	0	2	19		33	
$M25F0_6$	9	23	0	7	27	3	32		69	
$M25F25_1$	18	11	0	8	20	6	29		63	
$M25F25_2$	10	18	3	6	12	16	28		65	
$M25F25_3$	13	8	7	2	30	14	21	$\bar{x}=17.67$	74	$\bar{x}=55.83$
$M25F25_4$	7	4	2	0	31	9	11	($\pm$ 9.99)	53	($\pm$15.08)
$M25F25_5$	2	2	7	0	17	3	4		31	
$M25F25_6$	8	5	0	2	26	8	13		49	
$M0F50_1$	5	7	0	1	13	6	12		32	
$M0F50_2$	0	0	0	1	32	21	0		54	
$M0F50_3$	4	7	2	0	17	10	11	$\bar{x}=14.00$	40	$\bar{x}=48.67$
$M0F50_4$	14	0	9	0	9	26	14	($\pm$12.28)	58	($\pm$11.17)
$M0F50_5$	3	7	0	0	18	19	10		47	
$M0F50_6$	26	11	5	1	11	7	37		61	
$M50F0_1$	6	13	7	2	30	10	19		68	
$M50F0_2$	0	4	0	4	43	9	4		60	
$M50F0_3$	12	6	1	6	12	6	18	$\bar{x}=12.17$	43	$\bar{x}=48.50$
$M50F0_4$	11	8	1	2	21	9	19	($\pm$ 7.52)	52	($\pm$ 14.10)
$M50F0_5$	0	3	0	0	17	11	3		31	
$M50F0_6$	8	2	0	1	19	7	10		37	
$M50F50_1$	0	4	1	1	38	8	4		52	
$M50F50_2$	0	0	0	2	15	13	0		30	
$M50F50_3$	2	0	2	0	11	14	2	$\bar{x}=8.33$	29	$\bar{x}=37.33$
$M50F50_4$	2	1	0	1	9	13	3	($\pm$10.63)	26	($\pm$12.36)
$M50F50_5$	13	0	9	0	6	5	13		33	
$M50F50_6$	20	8	0	4	14	8	28		54	

servation indicates that the dose/production, and particularly the dose/naupliar survival relationships may be reasonably explained through exponential inactivation; that is, the surviving naupliar population and the total number of eggs produced decrease at an exponential decay rate with increasing fluence. The data, therefore, may fulfill basic target theory requirements. The "target theory" has been proposed as a mechanism to explain single cell survival following irradiation. It can be expressed simply as:

$$S = e^{-kD}, \quad (1)$$

where S is the fraction of the surviving population (production or naupliar survival) following exposure to dose D, e is the base of natural logarithms, and k is the proportionality constant (rate of inactivation). The theory is not unreasonable, for many biological systems appear to respond to radiation according to kinetics predicted by target theory.

Data for the number of eggs and the number of living nauplii produced by irradiated parents was subjected to two-way ANOVA between replications (Experiments 1 and 2) and treatments (M0F0–M50F50). No significant difference was found between the two fecundity experiments ($P > 0.05$). Therefore, data for both runs was combined for further analysis. A highly significant difference

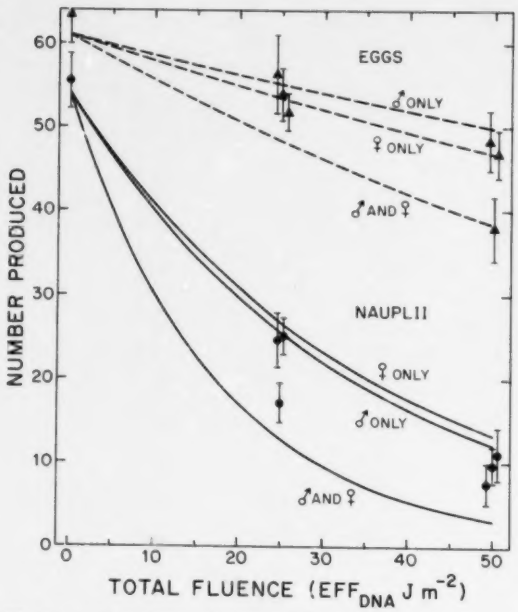

Fig. 2. *Acartia clausii.* Multiple curvilinear regression analyses illustrating production of eggs and living nauplii by mating pairs. Curves predict regression responses for the following equations:

$$(\text{eggs}) = 61.04\, e^{-(0.004\, D_m + 0.005\, D_f)}$$

and

$$(\text{nauplii}) = 54.03\, e^{-(0.030\, D_m + 0.028\, D_f)},$$

when only the male parent was irradiated ($D_f = 0$), only the female parent was irradiated ($D_m = 0$), or when both parents were irradiated equally ($D_m = D_f$). Data points represent sample mean (± SE); $n = 84$ for each triad of curves

was determined in the response of the organism to the different treatments ($P < 0.001$).

Multiple curvilinear regression analysis was used to apply target theory to the combined fecundity data. The resulting models, depicted in Fig. 2, provided the following relationships between UV-B exposure and fecundity for separate mating pairs of copepods:

$$(\text{eggs}) = 61.04\, e^{-(0.004\, D_m + 0.005\, D_f)} \quad (2)$$

and

$$(\text{nauplii}) = 54.03\, e^{-(0.030\, D_m + 0.028\, D_f)}, \quad (3)$$

where D_m represents the DNA-weighted level of exposure for the parental males and D_f is a comparable measurement for the parental females. The data fit was significant for both models ($P < 0.01$). Analysis further revealed that neither the number of eggs produced nor the number of nauplii developing from each mating pair was significantly affected by whether it was the male or the female parent which was irradiated. This non-significant difference ($P > 0.05$) between inactivation constants suggests the male and female reproductive stem cells may be equally sensitive to UV-B radiation.

Table 5 presents the results of two separate experiments testing the survival to maturity of 30 non-irradiated nauplii

Table 5. *Acartia clausii.* Survival data from two experiments for non-irradiated nauplii ($n = 30$ per cross type per experiment) produced by organisms used for mating crosses

Parental cross type	% nauplii surviving to maturity
M0F0	86.5± 9.2
M0F25	83.5± 9.2
M25F0	63.5± 4.9
M25F25	70.0± 9.9
M0F50	68.5± 2.1
M50F0	83.0± 14.1
M50F50	68.5± 2.1

collected from the organisms used, for each of the 7 types of mating crosses. Two-way ANOVA was performed with the original data, testing the difference in survival between parental type and replications. Neither *F*-statistic was significant ($P > 0.05$), indicating that previous exposure of parental stock did not significantly affect the subsequent survival capability of the offspring.

Discussion and Conclusions

Sublethal effects exhibited by *Acartia clausii* following an acute UV-B exposure have been documented previously (Karanas *et al.*, 1979). A marked decrease in the number of second-generation organisms produced per surviving adult suggested that even when a copepod survived exposure to UV-B radiation its ability to reproduce was impaired. The present study more closely investigates this effect by exposing virgin organisms to low exposure levels and then rearing the progeny of mating crosses between survivors.

The results demonstrate that sublethal UV-B exposure of virgin late copepodites significantly affects survival and the ability of both sexes to reproduce upon attainment of sexual maturity. Reproductive impairment appears to be a more sensitive measure of UV-B stress than survival. The irradiation of either one parent or both parents effectively reduces the total egg and living nauplii production, the latter seemingly the more sensitive bioassay. There also appears to be an additive interaction when both parents receive the UV-B treatment. When both parents received a specific treatment, similar results were obtained as when a single parent received a dose at a level equivalent to the sum of the treatments of the irradiated pair (e.g. an exposure level of 25 eff$_{DNA}$ J m^{-2} for each parent produced about the same results as an exposure of 50 eff$_{DNA}$ J m^{-2} for the male parent mated to a non-exposed female).

Presented in Table 6 are the depths at which a daily fluence of 25 and 50 eff$_{DNA}$ J m^{-2} would be expected to occur in the summer at 44°35′N for the four water types discussed by Smith and Baker (1979). The horizontal and vertical distribution patterns exhibited by *Acartia clausii* in nature determine the severity of the response to solar UV-B radiation. *A. clausii* is predominantly a coastal and estuarine copepod, ubiquitous in the northern hemisphere

Table 6. Predicted depths at which 25 and 50 eff_{DNA} J m^{-2} d^{-1} would be expected to occur in summer at Latitude 44°35′N for the 4 water types discussed by Smith and Baker (1979). This assumes a daily surface dose of 107 eff_{DNA} J m^{-2} d^{-1} (July 1) at a constant irradiance (Green *et al.*, 1980). Water types physically described as: (1) clearest open ocean water containing minimum amounts of chlorophyll *a* (0.025 mg m^{-3}) and dissolved organic matter (DOM; Sargasso Sea 65°41′W; 25°43′N); (2) clear ocean water with low chlorophyll *a* concentration (0.05 mg m^{-3}) from middle of Gulf of Mexico (89°W; 25°N); (3) moderately productive ocean water (0.5 mg chlorophyll *a* m^{-3}) from model of Smith and Baker (1978); (4) moderately productive ocean water (0.5 mg chlorophyll *a* m^{-3}) containing abnormally high concentrations of DOM relative to chlorophyll from coastal waters northwest of Tampa, Florida, USA (83°06′W; 28°38.5′N)

Water type	Effective attenuation coefficient (K_{DNA})	Effective depth (m)	
		25 eff_{DNA} J m^{-2} d^{-1}	50 eff_{DNA} J m^{-2} d^{-1}
1	0.163	8.92	4.67
2	0.197	7.38	3.86
3	0.377	3.86	2.02
4	0.920	1.58	0.83

(Carrillo *et al.*, 1974). However, certain species have been located in offshore waters. Cross and Small (1967) found *A. danae* present throughout the year in waters farther than 185 km off the Oregon coast. Other investigators (Davis, 1949; Frolander, 1962; Fleminger, 1964) report similar findings for the Washington, Oregon, and most of the California coastline. Deevy and Brooks (1977) recorded species of *Acartia* in the 0 to 500 m depth range of the Sargasso Sea.

Numerous vertical distribution studies for *Acartia* species appear in the literature. Vertical patterns of distribution do not appear constant throughout the organisms' life cycle. Several workers report an abundance of nauplii of *Acartia* spp. predominantly near the surface of the ocean (Barnes and Marshall, 1951; Barlow, 1955; Cameron, 1957; Furuhashi, 1976; Landry, 1976; Bakker *et al.*, 1977). Copepodites and adults, however, do not always follow the same pattern. Many field studies reveal a greater number of *A. clausii* and *A. tonsa* late copepodites and adults in bottom samples during the day and cite evidence of vertical migration (Jacobs, 1968; Anraku, 1975; Furuhashi, 1976; Landry, 1976). Landry describes a similar activity for all copepodites of *A. clausii*.

In contrast to references suggesting a benthic habitat for *Acartia* spp. by day, other workers have described numerous copepodites and adults in surface waters throughout the day. Cameron (1957) investigated the horizontal and vertical distribution of many pelagic copepods in the Queen Charlotte Islands off British Columbia, Canada: all life stages of *A. clausii* were living near the surface, occurring immediately below the surface in most areas. Barlow (1955) investigated the distribution of zooplankton in a small estuary near Woods Hole, Massachusetts, USA: although a greater concentration of *A. tonsa* early copepodites was found in bottom samples, densities of 10^5 individuals m^{-3} were measured at the surface. Deevy (1960) described *A. tonsa* as the most abundant copepod in the surface waters of the Delaware Bay region, USA. Bakker *et al.* (1977) described the copepod biomass in Lake Veere in the Netherlands: maximum copepod densities occurred at different depths during the summer of 1974; in July, high numbers of *A. tonsa* adults were sampled at the surface (max. 3.2×10^5 individuals m^{-3}); this magnitude of surface density was not recorded for any day other than 18 July, although for all sampling days some percentage of the sample population was always at the surface; for the months of June through August this percentage ranged from 5 to 42%.

From the literature it appears likely that, at some early stage of development, *Acartia* spp. may regularly inhabit the surface waters during the day. Although this is not always the case for copepodites of the age used in the present study, all individuals irradiated were collected between the surface and 1 m at midday.

As a result of wide geographical distribution, *Acartia* spp. occur in waters of widely varying optical properties. Table 6 illustrates that exposure levels used in this experiment to produce sublethal, reproductive damage in *A. clausii* can be expected to occur in one day during the summer months at some depth for all water types discussed. Certain water types described by Smith and Baker (1979; see legend to Table 6 of present paper) are more applicable to the distribution of *Acartia* spp. than others. The organism's coastal and estuarine affinity, as well as its vertical distribution, should be considered when evaluating possible UV-B impact in each water type.

Daily egg-laying rates for *Acartia* spp. cited in previous literature (Johnson and Miller, 1973; Landry, 1976; Parrish and Wilson, 1978) are substantially higher than what was encountered for control organisms in the present experiment (Tables 3 and 4). This could result from the numerous additional variables introduced throughout the lengthy experimental procedures employed in this study. The irradiation procedure required extensive handling of organisms and exposure to a 10C° temperature variation over a relatively short period of time. Eggs were not removed from each male-female pair and counted every day, a difference in procedure which introduced variables not encountered by the other investigators. Landry (1976) reported that *A. clausii* adults acted as voracious cannibals, especially on young nauplii, during periods of depleted food conditions. Lonsdale *et al.* (1979) also discovered this phenomenon with *A. tonsa;* however, they were found to feed upon young regardless of the food concentration. Possible egg and nauplii loss through adult grazing cannot be disregarded for the present experiment. Natural mortality and disintegration of nauplii hatched early in the 7 d period before the final count could be factors in the apparent reduced productivity. Also, rearing temperatures in the laboratory, although within the optimal range, were approximately 3C° cooler than temperatures in the field. With no acclimation period, this temperature drop may have had some effect on fecundity.

It is likely that the experimental procedure as well as UV-B stress affected the reproduction of the experimental organisms. An interaction between these two variables should be considered in interpretation of all results. No photorepair studies were performed during the course of these experiments. It is probable that maximum photorepair was not achieved by the irradiated copepods, for the relatively low visible irradiance used to inhibit phototactic behavior would promote less photoreactivation than sunlight. Further study would be required to determine the proper irradiance level for maximum repair.

There has been no *in situ* investigation dealing with effects of solar UV-B on *Acartia* spp. However, where it was previously questionable whether enough solar UV-B was striking the surface of the ocean to damage the adult copepod population, it is now obvious that fluence levels below those required to affect the organism's survival could alter the population on the basis of effects upon reproductive capability.

Discretion is advised when relating the results presented in this paper to a natural body of water. Variables such as attenuation by the water column, vertical position of the organisms, mixing, behavioral responses, and photorepair mechanisms require careful consideration. Utilization of the DNA action spectrum as a sensitivity function for a marine copepod also merits caution. The DNA action spectrum is most sensitive to fluctuations in shorter wavelengths of UV-B radiation. This is the region of the UV-B waveband that is affected most severely by fluctuations in stratospheric ozone concentration. If *Acartia clausii* were to respond to longer wavelengths of radiation at a level greater than that predicted by the DNA sensitivity function, the impact of enhanced levels of solar UV-B radiation resulting from ozone depletion might be lessened. Finally, although near-surface organisms may be impacted by solar UV-B radiation, the effect on the total population distributed throughout the water column is still open to question.

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